

WING FORM AND GENE FUNCTION IN NINE GENOTYPES OF *DROSOPHILA MELANOGASTER*¹

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We have reported previously on a number of experiments on the vestigial locus of *D. melanogaster* in which marked changes of wing form were studied (Harnly, 1930a, b, 1936, 1940a, b, 1941; Harnly and Harnly, 1935, 1936). During the course of those experiments a number of minor wing margin defects were observed at certain temperatures in otherwise constant normal phenotypes. Other genotypes, approaching the wild phenotype in only one part of the viable temperature range, had major or minor wing margin variations in those nearly normal wings. A larval thermolabile period in wing development at high temperatures for the mutant vestigial of *D. melanogaster* has been reported by several workers (Harnly, 1930b, 1936; Li, 1936; Stanley, 1931, 1935). The pupal period of vestigial has proved refractory at high temperatures. The following experiments were: (a) an attempt to find a pupal thermolabile period in vestigial; (b) a study of the effects of less extreme alleles and modifiers on wing margin variation; and (c) the determination of the temperature-effective-period of these wing margin variations in preparation for an histological study of wing development during the larval and pupal periods.

TECHNIQUE

Unless otherwise specified, the stocks used in these experiments have all been backcrossed in single pair matings for many generations to males from our inbred vestigial stock (single pair sib matings). These strains should therefore differ only by the mutant genes indicated. Mass matings of the genotypes examined laid eggs for a one or two hour interval on small trays of banana-agar media painted with a heavy yeast suspension. These trays were incubated at 25° C. for 24 hours. Ten recently hatched young larvae were then transferred to each 1 × 4 inch vial containing the yeasted customary banana-one per cent agar medium. This

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concentration of larvae per unit area is well below the point where crowding affects the wings. The larvae transferred were selected for uniform size. This technique insures uniformity of larval age as any eggs that have been held back by the females after fertilization will have hatched earlier and all these larger larvae were discarded. The high degree of accuracy of temperature control in our incubators has been discussed previously (Harnly, 1936). Vials of larvae transferred from a high to a low temperature (16°) were placed first at 10° for thirty minutes to lower rapidly the temperature of the food mass. The treatment of the larvae is described below for each genotype. The wings of all emerged flies were examined under $15\times$ magnification.

THERMOLABILE PERIOD FOR VESTIGIAL, DIMORPHOS VESTIGIAL, AND WING-MARGIN GENES

One hundred and forty vials of newly hatched vestigial larvae were placed at 32° until they had completed a total of 120 hours of development from the time the eggs were laid. Seventy vials were then transferred to 25° and the other seventy vials to 16° for the completion of development. The wing size and phenotype ("notched") of the flies emerging at 25° and at 16° were the same and similar to those previously reported for transfers at this time interval from 32° to 25° (see Harnly, 1936 for wing drawings and measurements for 32° to 25°).

Vials containing newly hatched vestigial larvae were placed in 32° incubators. Beginning shortly before the onset of puparium formation these vials were examined hourly and recently formed puparia isolated. One-fourth of these were immediately placed at 25° for the completion of development and emergence. The second quarter were placed at 16° for all subsequent development. The third quarter remained at 32° for an additional 24 hours and were then placed at 16° . The fourth group were transferred to 16° where they remained for six days and were then transferred to 25° for emergence. The results in all four groups were identical with each other and with those described above. There was only one significant difference between those transferred at 120 hours and at puparium formation. In the latter animals the wings were much more frequently bilaterally symmetrical in size and general form.

Pupation is extremely difficult at 32° but occurs normally at 28° , 11 hours $\pm$ 30 minutes after puparium formation. Vials containing recently hatched vestigial larvae were placed in 32° incubators. At the time of puparium formation regular inspections were made and puparia formed within the hour were isolated and placed in 28° incubators. The controls remained at 28° for the completion of development and the

emergence of the imagos. After 12 hours at 28° the rest of the vials were returned to 32° where one third remained for 12 hours, one third for 18 hours, and one third for 24 hours. All of these pupae were returned to 28° for the completion of development because homozygous vestigial flies do not emerge at 32°. The results obtained with these three sets of experimental animals were identical with their controls, with those obtained for transfers in this work from 32° to 25° and 16°, and with those previously reported for transfers from 32° to 25° for similar time intervals (Harnly, 1936).

We have reported previously on temperature-effective-period tests of the reciprocal type (Harnly, 1930*b*). In those experiments the development of the vestigial animals began at 29°, one degree below the critical temperature for the increase in wing size (Harnly, 1930*a*). The controls remained at 29° for total development. Groups of experimental animals were transferred at intervals of 24, 48, and 72 hours after egg laying, puparium formation, pupation, and 24 and 48 hours after pupation from 29° to 31° for the completion of development and the emergence of the imagos. Again the thermolabile period ended at the time of puparium formation. From all the experiments reported above it is evident that in the case of the vestigial genotype there is no thermolabile period for the development of wing form during the pupal stage at high, intermediate, or low temperatures.

All of the varied experiments on the vestigial genotype reported above were repeated with the dimorphos vestigial genotype. (Dimorphos is a sex-linked recessive modifier of vestigial.) No temperature response occurred during the pupal period either at high, intermediate, or low temperatures, hence the temperature-effective-period is restricted to the larval stage in this genotype. (For a description of dimorphos vestigial and its general response to temperature see Harnly and Harnly, 1935.)

In previous work with the stock of vestigial-pennant (a reverse mutation from vestigial to a recessive "wild") that had been repeatedly back-crossed to the inbred vestigial strain perfect wild-type wings were produced at 16° on part of the flies; animals developing at all higher temperatures had minor nicks on both wings (Harnly and Harnly, 1936).

Vials of newly hatched larvae were placed at 25° to determine whether this nicking is thermolabile prior to or after puparium formation. Control vials remained at 25° for the completion of development and emergence of the adults. Experimental animals were transferred to 16° at 96 hours after egg laying and at the hour of puparium formation which took place 110–130 hours from egg laying, the bulk of the puparia being formed between 114 and 122 hours. There were no perfect wings on 477 control males and 445 control females nor on 708 males and 552 females

transferred to 16° the hour they had formed puparia. The 96-hour shift to 16° gave 17 per cent of the males with one perfect wing and 3 per cent of them had both wings perfect; 15 per cent of the females had one perfect wing and an additional 6 per cent had both wings perfect. The data demonstrate that the presence or absence of minor nicks in this genotype is determined by the temperature experienced during the larval period prior to puparium formation. Since the frequency of normal wings was not as high as that obtained for total development at 16° the temperature-

TABLE I

Wing characteristics of stock *b vg^P*, reared at temperature indicated, 2 trials.

O—number of flies neither wing showing characteristic indicated, L—left wing shows, R—right wing shows, LR—both wings show

°C.	Wing Variation	Males				Females			
		O	L	R	LR	O	L	R	LR
16	Margin Normal				340				310
25		38	68	69	162	236	47	39	45
30		124	87	88	75	181	42	27	12
30	Margin Normal -V		7	16			16	10	1
25	Extra X-Vein II-III	206	50	49	32	263	51	44	9
30	Extra X-Vein II-III	230	51	59	34	209	16	23	14
	Extra X-Vein III-IV	340	10	23	1	237	14	11	
	Extra X-Vein II-III and III-IV	365	5	3	1	256	2	3	1
16	Delta	258	35	37	8	219	35	41	15

effective-period must begin prior to 96 hours of total development and end after 96 hours but no later than the time the puparia are formed.

WING-MARGIN AND VEIN GENES

It had been observed that the original black vestigial-pennant stock threw a good many males and females with normal wing margins at 25°. Black vestigial-pennant animals were reared at 16°, 25°, and 30°. The data are shown in Table I. They differ markedly from those obtained from the backcrossed vestigial-pennant flies. Both wings of all black vestigial-pennant flies had normal margins at 16° and a fairly large

proportion of the flies had at least one wing with a normal margin at 25° and 30°. The difference in the results with the two vestigial-pennant stocks indicated that the marginal nicks must be due, at least in part, to one or more other loci.

Green and Oliver (1941) have reported "that a larval temperature of 27° is sufficient to increase significantly the wing length of black-vestigial and yellow-vestigial males and females. . . . No such effect was noted among vestigial and vestigial-sooty individuals." The differences between the wings in comparative samples of these genotypes were not only significant but actually profound. We have tested the black vestigial and the yellow vestigial stocks carried in our laboratory at 25°, 27°, 28°, 29°, 30°, 31°, and 32°. All of the males and the females reared at the intervals from 25° through 29° had typical "vestigial" wings. Our previously reported changes in phenotype at 30° and above were also observed in these two stocks. Evidently, these pigment genes do not influence the phenotype of homozygous vestigial and Green and Oliver are dealing with some other modifier of vestigial lowering markedly its critical temperature as does *dimorphos* (Harnly and Harnly, 1935). Mr. Green informs me that in view of certain internal evidence in his stocks, this interpretation is probably correct.

Four new conditions were observed in the wings of the black vestigial-pennant stock. (1) The distal end of the V-longitudinal vein was absent at 30° in 8-10 per cent of the wings with perfect margins. (2) An extra cross-vein between the II and III-longitudinal veins proximal to and near the anterior cross-vein was present at 25° in at least one wing on 40 per cent of the males and 28 per cent of the females. At 30° this extra cross-vein appeared on the wings of 40 per cent of the males and 20 per cent of the females. (3) An extra cross-vein between the III and IV-longitudinal veins was seen at 30° on 9 per cent of the males and the females; and an occasional animal had both extra cross-veins on the same wing. There was no obvious relation between a perfect or imperfect wing margin and the appearance of these extra cross-veins. (4) A delta appeared at the junction of the posterior cross-vein with the IV-longitudinal vein in at least one wing of approximately 25 per cent of the males and the females at 16°.

TEMPERATURE-EFFECTIVE-PERIODS OF MARGIN NICKS, EXTRA CROSS-VEINS, DELTA, AND INCOMPLETE V-VEIN

Transfers were made from 30° to 16° to determine the period during development when these conditions are thermolabile. The results are

shown in Table II. The data indicate that the temperature-effective-period for marginal nicks (all animals normal at 16°) begins at approximately 60 hours in both sexes and ends for the females at about 90 hours and for the males around 96 hours of total development. Mr. Herbert J. Levin (unpublished) has found that the mean time from egg laying of the molt between the second and the third instar in our inbred vestigial stock is 68 hours at 25° and 59 hours at 31°. Evidently the thermolabile period for marginal nicks in the black vestigial-pennant stock begins with the molt from the second to the third instar and terminates before puparium formation. The temperature-effective-period for both types of extra cross-veins (appear at 30°) must be during the pupal period since these veins were not found in the wings of animals transferred from 30° to 16° earlier than 120 hours. The delta (shows at 16°) appeared with

TABLE II

Stock b vg^p, frequency of flies with normal wing margins, animals developing at 30° are transferred to 16° at the intervals indicated, 2 trials.

Legend O, L, R, LR as in Table I

Wing Variation	Age at Transfer	Males				Females			
		O	L	R	LR	O	L	R	LR
Margin Normal	60 Hours		1		123		3	4	207
	72 Hours		3	3	242	5	26	40	278
	78 Hours		3	5	148	30	47	34	165
	84 Hours	2	8	14	70	64	20	24	66
	90 Hours	13	11	16	14	93	19	19	7
	96 Hours	21	11	11	19	111	19	16	24
	120 Hours	19	36	28	62	136	34	38	20

the usual frequency in the groups of flies from all of these time intervals and would seem to be determined during the pupal period.

The reciprocal transfers from 16° to 30° of individuals isolated at the hour of puparium formation constitutes a critical test of the above interpretations. Both wings had normal margins on all the males and the females transferred from 16° to 30° immediately after puparium formation. Furthermore, some 60 per cent of the males and 54 per cent of the females had the extra cross-vein between the II and III-longitudinal veins on one or both wings. Twenty per cent of the males and 22 per cent of the females had an extra cross-vein between the III and IV-longitudinal veins on one or both wings. Both extra cross-veins were observed in the same wing on one or both wings of 41 per cent of the males and 34 per cent of the females. All possible combinations of these

three conditions were seen on the two wings of different individuals. These reciprocal transfers demonstrate conclusively that the entire temperature-effective-period for the presence or absence of the wing margin nicks is during the third larval instar prior to puparium formation and that the critical period for the extra cross-veins is restricted to the pupal stage. The delta (appears only at 16°) did not show in the wings of any of these animals and therefore has its thermolabile stage restricted to the period following puparium formation. Even with complete development at 30° the frequency of the incomplete V-longitudinal veins is low. Consequently the data from the reciprocal transfers is inconclusive but suggests that the temperature-effective-period for this character follows puparium formation.

BLACK VESTIGIAL-PENNANT/VESTIGIAL (UNRELATED)

The marked difference between the data of the vestigial-pennant (derived from repeated backcrossing to inbred vestigial) and the black vestigial-pennant stocks on wing margin nicks, delta, extra cross-veins, and V-longitudinal vein, indicate that these two stocks of vestigial-pennant must differ by one or more other wing determining genes. Experiments were performed to determine something of the number and nature of these other wing genes.

We have shown previously that the heterozygote between the inbred vestigial stock and the vestigial-pennant stock that had been repeatedly backcrossed to it produced deeply notched wings at 32° and "cut," "antlered," and "strap" wings at lower temperatures (Harnly and Harnly, 1936). The original black vestigial-pennant stock was crossed to a non-inbred vestigial stock. This heterozygote produced rather large numbers of males and females at 32° with both wings having perfect margins. The flies with imperfect wing margins at 32° had only very minor nicks in the distal end of the wings between the III and IV-longitudinal veins. Extra cross-veins appeared fairly frequently in the wings of the flies reared at 32°. At lower temperatures the wings were markedly defective and similar to those reported previously (Harnly and Harnly, 1936). The difference in the wings produced at 32° by these two identical vestigial locus heterozygotes must be due to differences in other loci; one set of modifiers producing deeply "notched" wings, the other perfect wild-type wings.

DOMINANT-RECESSIVE DETERMINATIONS

Reciprocal crosses were made between the vestigial-pennant (derived from repeated backcrossing to inbred vestigial) and the black vestigial-pennant stocks to determine whether the modifiers causing the delta, extra

cross-veins, an incomplete V-longitudinal, and margin nicks are dominant or recessive, sex-linked or autosomal. The offspring were reared at 16°, 25°, and 30°. The results are shown in Table III.

TABLE III

Variation of wing characteristics, F₁ of reciprocal crosses reared at temperature indicated, 2 trials. Legend O, L, R, LR as in Table I

	Wing Variation	°C.	Males				Females			
			O	L	R	LR	O	L	R	LR
b vg ^p ♂ × b ⁺ vg ^p ♀	Margin Normal	16	1	16	13	129	2	18	15	153
		25	116	11	18	3	103	26	16	6
		30	56	72	72	112	93	32	28	205
	Delta	16		3	1			12	6	1
	Extra X-Vein III-IV	30							1	
b ⁺ vg ^p ♂ × b vg ^p ♀	Margin Normal	16	2	7	7	170	2	9	8	186
		25	59	34	42	31	78	33	26	10
		30	5	45	43	396	36	33	31	344
	Delta	16		1	1			9	6	
	Extra X-Vein III-IV	30		3	3				3	

An extra cross-vein between the II- and III-longitudinal veins in one or both wings was found frequently on the black vestigial-pennant males and the females at 25° and 30° (Table I). This extra cross-vein was not observed on any of the offspring from the reciprocal crosses at either 25° or 30°. The extra cross-vein between the II- and III-longitudinal veins is evidently due to an autosomal recessive.

An extra cross-vein between the III- and IV-longitudinal veins was observed on some of the black vestigial-pennant males and females reared at 30° (Table I). The data from the reciprocal crosses seems to indicate that this extra cross-vein is controlled in part by a semi-dominant sex-linked gene carried in the X-chromosome of the black vestigial-pennant stock. However, its expression must be modified by an autosomal gene in the backcrossed vestigial-pennant stock since the frequency in the sons out of black vestigial-pennant by vestigial-pennant is markedly reduced.

The evidence is inconclusive on the mode of inheritance and dominance of the gene occasionally causing an incomplete V-longitudinal vein at 30°.

The delta appears with a lower frequency in both sexes among the offspring of the reciprocal crosses and is probably due to an autosomal semi-dominant not finding expression as frequently in the heterozygote as in the homozygote. In the homozygote it appears significantly more frequently in the females than in the males. In the heterozygote this sexual dimorphism in frequency is profoundly increased.

Clear-cut conclusions cannot be drawn on the mode of inheritance of the margin gene or genes. The data of the males seem to indicate that the X-chromosome of the black vestigial-pennant stock carries a gene tending toward normal margins. This is indicated by the higher percentage of males with normal margins from black vestigial-pennant mothers than the reciprocal males with vestigial-pennant mothers. However, the sisters of the first type also have a higher frequency of normal wings than the reciprocal females. These two sets of females should have identical genotypes. Likewise the change in frequency with temperature is disconcerting. A fair percentage of the vestigial-pennant (derived from repeated backcrossing to inbred vestigial) flies have normal wings at 16° but produce no normal wings at higher temperatures. The black vestigial-pennant stock produces only normal wings at 16° and at higher temperatures produces a steadily decreasing proportion of normal wing margins. The reciprocal heterozygotes produce the lowest percentage of normal margins at 25° with markedly higher percentages of normals at both 16° and 30°. Since the two homozygotes have similar temperature responses, a shift in dominance with temperature in the heterozygote corresponding to that found in vestigial/vestigial-pennant (Harnly and Harnly, 1936) does not seem possible, i.e., this difference cannot be due to a change having taken place in the vestigial-pennant gene in one of these two stocks. Probably more than one locus, other than the vestigial locus, is concerned in the production of marginal nicks. It may be that these genes are the *domini* genes of Goldschmidt but it would take additional work to determine this point (Goldschmidt, 1937*a*, *b*; Goldschmidt and Höner, 1937).

PROOF OTHER LOCI CAUSE DIFFERENCES BETWEEN VESTIGIAL-PENNANT STOCKS

A critical proof that the differences between these two vestigial-pennant stocks (extra cross-veins, degree of margin nicking, delta, etc.) were not due to the vestigial locus would come from a transfer of these differences from one stock to another stock. The black vestigial-pennant line was crossed to an unrelated vestigial strain not used in any of our previous work. From the F₁ hybrids two new stocks were developed.

In the first case black vestigial-pennant/vestigial females were backcrossed to black vestigial-pennant males and through forty generations the hybrid females were backcrossed to the original black vestigial-pennant strain (single pair matings). Homozygous vestigial was then obtained by inbreeding the forty first generation. This new vestigial line should carry the I, III, and IV chromosomes and through cross-overs practically the entire II-chromosome of the black vestigial-pennant stock, differing from it only at the vestigial locus and perhaps a few adjacent loci. In the second case the F_1 hybrid females and their daughters through forty generations were backcrossed to males from the unrelated vestigial stock (single pair matings). Inbreeding the forty first generation produced a new vestigial-pennant line which likewise should have had the genes replaced at all loci save the vestigial locus. These two new stocks were then tested.

Larvae of the new vestigial stock were reared at 32° until puparium formation and then transferred to 30° for the completion of development and emergence. A very high percentage of these flies had an extra cross-vein between the II- and III or III- and IV-longitudinal veins (or both) on one or both wings. The presence or absence of these extra cross-veins is not dependent on the gene at the vestigial locus. They are therefore due to other independent loci. The wing phenotype and size were similar to that reported previously for vestigial reared at 32° (Harnly, 1936). The only significant difference observed in wing shape and size was a tendency toward more nearly perfect wings. An occasional fly had only minor nicks or a small notch in the wing at the distal end between the III- and IV-longitudinal veins. This would indicate that the gene or genes for less nicking (normal margins) in the black vestigial-pennant stock had been transferred to the vestigial stock by the repeated backcrosses. It furthermore emphasizes the fact that the size and form of the wing are determined by temperature, the vestigial gene, and other loci as well; in other words, by the entire genotype *and* the environment. The delta at the junction of the posterior cross-vein with the IV-longitudinal vein reported above appears only in the wings of flies reared at 16° . Due to the small size of the vestigial wings at this temperature, it was impossible to test for the presence of this gene.

The new vestigial-pennant stock was tested for the replacement, by backcrossing, of the assumed genes. The data demonstrate that this substitution had taken place (Table IV). Some of these flies differed from the black vestigial-pennant stock by having defective wing margins at 16° but the percentage of flies with one or two normal wings was double that produced at 16° by the vestigial-pennant stock derived from repeated backcrosses to our inbred vestigial line. No perfect wings were

produced at 25°; but at 30° some wings with normal margins were produced. Here again the data differ from that obtained from the other backcrossed vestigial-pennant strain which produced normal wings only at 16° (Harnly and Harnly, 1936). At all three temperatures the data on the wing margin differ from that of the black vestigial-pennant stock (Table I) from which it had been derived by repeated backcrosses to the unrelated vestigial stock. Furthermore, the V-longitudinal vein was complete in all cases, the delta described for the black vestigial-pennant stock was not produced at 16°, and extra cross-veins did not appear at 25° and 30°. A different delta appeared at the junction of the posterior cross-vein with the V-longitudinal vein in the wings of some 14 per cent of the new type vestigial-pennant flies at 16°.

The data from the new vestigial and vestigial-pennant stocks demonstrate that: (1) the frequency of wings with normal or defective mar-

TABLE IV

Frequency of wings with perfect margins, $vg^P F_{40}$ strain, reared at temperature indicated, 2 trials. Legend O, L, R, LR as in Table I

°C.	Males				Females			
	O	L	R	LR	O	L	R	LR
16	21	37	47	222	22	48	50	319
25	357				418			
30	433	4	4		263	25	28	2

gins; (2) the presence or absence of a delta in the posterior cross-vein, and its location; (3) a complete or incomplete V-longitudinal vein; and (4) the presence or absence of extra cross-veins is due to genes independent from the vestigial locus.

DISCUSSION

In these experiments nine genotypes have been subjected to the rigidly controlled environmental variable, temperature. The characters examined were: (1) wing form variation from "vestigial," "strap," and "antlered" through "notched" and, in some cases, "nicked" to "normal" in four genotypes; (2) wing form normal with and without marginal nicks in six genotypes; (3) the presence or absence of extra cross-veins at two unusual points in the wings of four genotypes; (4) the presence or absence of a delta in the posterior cross-vein at either its junction with the IV- or V-longitudinal vein in three genotypes; and (5) an incomplete V-longitudinal vein in two genotypes. The phenotype of each genotype

varied with the temperature experienced during development. When several genotypes had a common character variation the phenotype found at one temperature was characteristic of a specific genotype and was produced at a different temperature by another genotype. In previous papers (Harnly, 1930*a, b*, 1936, 1940; Harnly and Harnly, 1935, 1936) we have reported on a similar temperature response of genes affecting the wing form, the arrangement of the postscutellar bristles, size and form of the balancers or halters, arrangement of the ommatidia, size and form of the eyes, and the presence or absence of wing margin nicks. It is evident that the gene or even the whole genotype does not solely determine the phenotype of the individual.

The genotype of the individual is fixed at fertilization. However, this does not determine the phenotype of the individual. At this moment the limits within which each character may be expressed are set by the genotype of the particular organism. For example, in the dimorphos vestigial stock the wings will never be smaller than "vestigial" and may be as perfect as "normal," the balancers may be minute vestiges or normal in size and form, the postscutellar bristles may point anteriorly or posteriorly, and the eyes may be "normal" or markedly abnormal. The exact expression within these limits is determined separately for each character by the environment within which each individual develops. For example, in dimorphos vestigial the wings, postscutellar bristle, and the eyes change phenotype at different critical temperatures (Harnly and Harnly, 1935). Among the environmental factors that have been demonstrated to act as determiners within the boundaries set by the genotype are: temperature density of population, type or kind of food media, humidity, etc., for *D. melanogaster*. These and other environmental factors have been found acting in other forms of animals and plants. The subjection of the individual successively to two or more points within the environmental variable during the character's critical stage (thermolabile period) results in a phenotype characteristic of neither environmental point. In the case of vestigial the phenotype is an intermediate, the exact expression depending upon the duration of exposure to the two temperatures in the critical period (Harnly, 1936, Fig. 7). The data presented here, in our earlier papers, and in the work of others demonstrates that the environment is affecting the response or action of the genotype in a critical period of some duration during the development of each character. The limits (extremes) for each character of the individual are fixed at fertilization by the genotype established and the phenotype of each individual is *developed* at a particular point within those limits as the resultant of the interacting forces of genotype and environment during the critical stages of ontogeny.

During the course of these experiments a marked difference was observed between several stocks in the frequency of wings with marginal nicks, and previously undetected wing mutants were seen in the original vestigial-pennant stock when it developed at extreme temperatures. The genes for extra cross-veins, delta, and an incomplete V-longitudinal vein were replaced with their normal alleles by repeated backcrossing of the original vestigial-pennant stock to an unrelated vestigial stock. This replacement also changed markedly the frequencies of "normal" vs. "nicked" wing. The reciprocal repeated backcrossing of the unrelated vestigial stock to the original vestigial-pennant line transferred the genes for extra cross-veins to the vestigial strain and, through the shift in what one may call "wing-margin" genes, changed the degree of defect in the wings of vestigial flies reared at a high temperature. Presumably the gene for the delta was also transferred to the vestigial line but due to the form of the wings of vestigial flies reared at 16° it was impossible to test for the presence of the delta gene. The reciprocal tests demonstrated that the two differently located extra cross-veins, the delta, and the frequency of "normal" vs. "nicked" wing margins are all due to a number of different loci and independent in their expression of the particular allele at the vestigial locus. The data presented here and in previous papers demonstrate that the various isolated vestigial stocks have accumulated different and undetected mutations affecting the wing margin, venation, etc. These effects can appear only when crosses transfer the mutant genes to other stocks having wings of normal form or when the vestigial strain happens to develop at an extreme temperature where the wing is more nearly complete and the new mutants can be expressed. Evolutionarily speaking the situation is analogous to a balanced lethal form in which recessive mutants may collect and never show until a rare cross-over occurs. Due to the action of the vestigial gene only the basal region of the wing is developed. Mutant genes affecting potential regions or structures of the wing, which are not developed by the vestigial flies at usual temperatures, may accumulate in the strain even though not expressed. Then an outcross or a single mutation at the vestigial locus will allow the entire group to be expressed suddenly at one moment in time. A similar situation in nature might explain some of the more abrupt and complex changes in evolution.

The loci used in these experiments fall into one or the other of two groups according to the character affected. (1) The alleles at the vestigial locus in the 2-chromosome, at the dimorphos locus in the 1-chromosome, and at the wing-margin loci all affect the amount and form of the wings present. The various combinations of alleles at the vestigial and dimorphos loci determine whether the degree of imperfection

in the wings shall vary from "vestigial" to "notched," or from "vestigial" to "nicked" or "normal," or from "strap" or "antlered" to "notched," "nicked," or "normal." The minor defects on these genotypes are all terminal defects of notching or nicking in the distal end of the wing. The particular combination of wing-margin alleles and the environment determine the range of variation from none to one or more "nicks" in the margins of wings that are otherwise "normal" or approaching normal. The nicks caused by these alleles may appear at any point in the wing margin. (2) The other loci reported here had no detected effect on the amount and form of the wings present. Their effects were on the venation within the wings. An autosomal recessive determined the presence of an exceptional cross-vein between the II and III-longitudinal veins in certain environments; an extra cross-vein between the III and IV veins was similarly determined by an apparently semi-dominant sex-linked gene; the presence in the proper environment of an exceptional delta in the junction of the posterior cross-vein with the IV-longitudinal vein was caused probably by an autosomal semi-dominant, and a fourth locus was associated with the appearance of an incomplete V-vein.

However, there was no consistent temperature response through the viable range within either the wing form or wing venation group of loci and alleles. The vestigial gene for wing form responds directly to increases in temperature but its allele vestigial-pennant responds inversely through the viable range (Harnly, 1930a; Harnly and Harnly, 1936). The results of the reciprocal crosses between the two vestigial-pennant stocks (Table III) and those on the new vestigial-pennant stock (Table IV) demonstrate that some genes for wing-margin defects find expression at low temperatures while others find expression at high temperatures. The same heterogeneous response was found for the group of loci affecting wing venation. One of the genes associated with the extra cross-veins finds expression at both intermediate and high temperatures while the other one is expressed only at high temperature. The gene for an incomplete V-longitudinal vein is expressed only at a high temperature but the one for delta breaks through only at a low temperature. The nature of the response through the viable temperature range is not a function of the type of character or even of the locus. The nature of the response to temperature is a function of the particular allele.

But the stage in development when the gene is thermolabile is not a function of the particular allele or of the locus. Nor would one expect it to be so if the temperature-effective-period has any relation to ontogeny. All of the genes affecting wing form had a more or less common thermolabile period during the larval stage. The data presented here and previously (Harnly, 1930b, 1936; Li, 1936; Stanley, 1935) demonstrate

that the temperature-effective-period at low, intermediate, or high temperatures for the vestigial gene begins at or shortly following the molt from the second to the third larval instar and ends, depending upon the temperature and the modifiers present, during that instar or at its close when the puparium is formed. Similarly the data reported here for dimorphos vestigial demonstrate that its critical period is restricted to the third larval instar. Unpublished data from combinations of alleles at the vestigial locus also demonstrate that the thermolabile period for wing form never ends later than the time of puparium formation. The data reported here for two different combinations of wing-margin genes (nicks) shows conclusively that the temperature-effective-period for both genotypes is restricted to the third larval instar and ends by the time the puparium is formed. One may conclude that genes affecting wing form have their temperature-effective-periods during the larval stage and that this period is ended with the formation of the puparium. Our accumulated unpublished data on many genotypes demonstrate that wing size, in part or whole, is an entirely different problem from wing form and will be considered elsewhere.

The thermolabile period for the genes affecting wing venation is different from that of the genes associated with wing form. The genes at the two loci for extra cross-veins, the locus for the delta, and the locus for the incomplete V-vein were not affected in their expression by the temperature (low, intermediate, or high) at which the larvae developed. The temperature experienced following the formation of the puparium determined whether the exceptional mutant character or the "normal" expression was developed. Mr. Melvin Green has informed me that the temperature-effective-period of vesiculated (vs. 29c) is also during the pupal period of development (unpublished).

Each of these two groups of loci has its own characteristic thermolabile period. Hence, the temperature-effective-period for a character is not a function of the locus or the allele present. This interval must be a function or characteristic of a definite stage in development when the action of either a gene or its product is thermolabile. It seems that both the inception and maximum duration of this period are determined by or linked to general ontogenetic processes. Work now well along in our laboratory on the development of the dorsal mesothoracic discs of three genotypes shows profound morphological changes taking place in these discs and their wing buds during the third larval instar. In studies on post puparium development of a number of mutants affecting venation, Waddington (1939, 1940) has found that minor variation such as extra cross-veins, incomplete veins, and delta effects are determined or differentiated morphologically during his P_2 stage (18-45 hours) after pu-

parium formation. Both his findings on the morphological determination of the wing veins and our tentative conclusions on the morphological development of the dorsal mesothoracic discs and the wing buds would support the conclusion that the thermolabile period for a particular locus is the time of growth and/or differentiation of the structure affected by the gene. Either a precursor material is formed by the gene at an earlier stage and its subsequent action during ontogeny is thermolabile at a specific period morphologically determinable or the action of the gene modifying the development of the character is direct, immediate, and thermolabile at the moment of growth and differentiation of that character. In either case the genetic thermolabile period is a morphological stage of growth and differentiation of the character. The gene or its products cannot affect the development of a character until that stage is reached by the organism, e.g., molt to third instar for wing form, at which that structure is determined, grows, or differentiates. The developing structure then becomes responsive to the action of that gene or the gene products. Our earlier studies have shown that both the rate and the duration of this response is determined by both the genotype and the environment of the individual. If, due to the nature of the allele and the environment, the action is not self-terminated then it is automatically stopped by the attainment of a definite stage in development, e.g., in some genotypes puparium formation for wing form. During this period either the genes causing the minor modifications in the wing venation have not been functioning or the structure is refractory to them or their products. It is as if there was nothing or not enough there yet on which an effect could be made. Following puparium formation the development and differentiation of the wing has progressed to a stage where the final detailed differentiation of the veins can take place. It is not until this stage in ontogeny that these wing vein genes or their products can influence the structure. The thermolabile period is the experimentally determined stage in the development of a given character when the genotype under examination can cut in on and modify the developing structure. Comparative studies now in progress both within the single genotype and between genotypes of the morphological changes during this period should elucidate the effects of the gene upon the mechanics of wing development.

SUMMARY

1. Nine genotypes, including a number of new mutants, affecting the wing form and the wing venation were found to vary in phenotype between 16° C. and 32° C.

2. The dominant-recessive relationships of these new mutants and the linkage group in some cases was determined.
3. The limits for the future phenotype of each character are demarked at fertilization by the genotype established. The phenotype of each individual is subsequently developed at a point within these extremes as the resultant of the interacting forces of the genotype and the individual's environment.
4. The nature of the response to temperature (positive or negative) throughout the viable range is a function of the particular allele.
5. All of the genes modifying the wing form were found to be thermolabile during the larval period prior to puparium formation.
6. All of the genes associated with minor modifications in the wing venation had their temperature-effective-period during the pupal stage following puparium formation.
7. The interval of the temperature-effective-period is not a function or characteristic of the allele or the locus but of the stage in development when the action of the gene or its products is thermolabile.
8. The rate of the response and the duration, if not the maximum, are determined by the allele and the environment.
9. The onset and maximum duration of this period are determined by definite developmental stages.
10. Apparently the development of the dorsal mesothoracic disc must progress to a certain point before the wing form loci become effective in further growth and differentiation of this structure and its wing bud. Loci causing minor modifications in the wing venation cannot become operative until much later when the final morphological differentiation of the wing is well advanced.

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